

## CLAIMS

1. A method of growing spermatogonial stem cells, which comprises growing spermatogonial stem cells by culturing  
5 the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF).
2. The method of growing spermatogonial stem cells of  
10 claim 1, wherein the above-described medium further contains at least one of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF).
3. The method of growing spermatogonial stem cells of  
15 claim 1 or 2, wherein the above-described medium further contains serum.
4. The method of growing spermatogonial stem cells of  
any one of claims 1 to 3, which further comprises using  
20 feeder cells.
5. The method of growing spermatogonial stem cells of  
any one of claims 1 to 4, which comprises using mammal-  
derived spermatogonial stem cells.
- 25 6. The method of growing spermatogonial stem cells of  
any one of claims 1 to 5, wherein the above-described glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto is contained at a concentration of  
30 0.5 to 50 ng/ml in the above-described medium.
7. The method of growing spermatogonial stem cells of  
any one of claims 1 to 6, wherein the above-described leukemia inhibitory factor (LIF) is contained at a

concentration of  $10^2$  to  $10^4$  units/ml in the above-described medium.

8. The method of growing spermatogonial stem cells of  
5 any one of claims 2 to 7, wherein epidermal growth factor (EGF) is contained at a concentration of 0.5 to 50 ng/ml in the above-described medium.

9. The method of growing spermatogonial stem cells of  
10 any one of claims 2 to 8, wherein the above-described basic fibroblast growth factor (bFGF) is contained at a concentration of 0.5 to 50 ng/ml in the above-described medium.

15 10. The method of growing spermatogonial stem cells of any one of claims 3 to 9, wherein the above-described serum is contained at a concentration of 0.1 to 5 (v/v)% in the medium at the start of cultivation of the above-described spermatogonial stem cells, and at a  
20 concentration of 0.1 to 20 (v/v)% in the medium after passage of the above-described spermatogonial stem cells.

11. The method of growing spermatogonial stem cells of  
25 any one of claims 4 to 10, wherein the above-described feeder cells are used by 4 weeks after the start of cultivation at latest.

12. Spermatogonial stem cells grown *in vitro* using the  
30 growing method of any one of claims 1 to 11.

13. A therapeutic agent for infertility containing the spermatogonial stem cells of claim 12.

14. A medium additive kit that comprises glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and at least one of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), and  
5 that is used as added to a culture medium for growing spermatogonial stem cells *in vitro*.
15. The medium additive kit of claim 12, which further comprises leukemia inhibitory factor (LIF).
- 10 16. The medium additive kit of claim 14 or 15, which further comprises serum.
17. Use of the spermatogonial stem cells of claim 12 for  
15 producing a therapeutic agent for infertility.
18. A therapeutic method for infertility using the spermatogonial stem cells of claim 12.
- 20 19. A method of producing a non-human animal that forms sperms derived from transplanted spermatogonial stem cells, which comprises the following steps:  
a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium  
25 containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);  
b) a step of transplanting the spermatogonial stem cells grown in step a) into a seminiferous tubule of an  
30 infertile non-human animal to obtain a non-human animal showing spermatogenesis derived from the spermatogonial stem cells.
20. A method of producing sperm, which comprises the

following steps:

- a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);
- b) a step of transplanting the spermatogonial stem cells grown in step a) into a seminiferous tubule of an infertile non-human animal to obtain a non-human animal showing spermatogenesis derived from the spermatogonial stem cells;
- c) a step of obtaining sperm from the non-human animal.

21. A method of producing an embryo derived from spermatogonial stem cells, which comprises the following steps:

- a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);
- b) a step of transplanting the spermatogonial stem cells grown in step a) into a seminiferous tubule of an infertile non-human animal to obtain a non-human animal showing spermatogenesis derived from the spermatogonial stem cell;
- c) a step of obtaining sperm from the non-human animal;
- d) a step of inseminating an ovum with the sperm to obtain an embryo.

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22. A method of producing non-human offspring derived from spermatogonial stem cells, which comprises the following steps:

- a) a step of growing spermatogonial stem cells by

culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);

5 b) a step of transplanting the spermatogonial stem cells grown in step a) into a seminiferous tubule of an infertile non-human animal to obtain a non-human animal showing spermatogenesis derived from the spermatogonial stem cells;

10 c) a step of obtaining sperm from the non-human animal;  
d) a step of inseminating an ovum with the sperm to obtain an embryo;

e) a step of transferring the embryo into an oviduct of a pseudopregnant female to obtain non-human offspring.

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23. A method of producing non-human offspring derived from spermatogonial stem cells, which comprises the following steps:

a) a step of growing spermatogonial stem cells by

20 culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);

b) a step of transplanting the spermatogonial stem cells  
25 grown in step a) into a seminiferous tubule of an infertile non-human animal to obtain a non-human animal showing spermatogenesis derived from the spermatogonial stem cells;

c) a step of naturally mating the non-human animal with  
30 a female to obtain non-human offspring.

24. A method of producing spermatogonial stem cells incorporating an extraneous gene, which comprises the following steps:

a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);

b) a step of introducing an extraneous gene to the spermatogonial stem cells grown in step a) to obtain spermatogonial stem cells incorporating the extraneous gene.

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25. A method of producing sperm incorporating an extraneous gene, which comprises the following steps:

a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);

b) a step of introducing an extraneous gene to the spermatogonial stem cells grown in step a) to obtain spermatogonial stem cells incorporating the extraneous gene;

c) a step of inducing spermatogenesis by transplanting the spermatogonial stem cells to a seminiferous tubule to obtain sperm incorporating the exogenous gene.

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26. A method of producing a transgenic non-human animal, which comprises the following steps:

a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);

b) a step of introducing an extraneous gene to the spermatogonial stem cells grown in step a) to obtain

spermatogonial stem cells incorporating the extraneous gene;

- c) a step of inducing spermatogenesis by transplanting the spermatogonial stem cells to a seminiferous tubule<sup>5</sup> to obtain sperm incorporating the exogenous gene;
- d) a step of inseminating an ovum with the sperm to obtain a transgenic non-human animal.

27. The production method of claim 26, wherein the<sup>10</sup> transgenic non-human animal is a knockout non-human animal.